

Please type a plus sign (+) inside this box →

11/17/00

No fee

PTO/SB/05 (08-00)

Approved for use through 10/31/2002 OMB 0910-0592
U.S. Patent and Trademark Office, U.S. DEPARTMENT OF COMMERCE
Box Patent Application, Washington, DC 20231

Under the Paperwork Reduction Act of 1995, no persons are required to respond to a collection of information unless it displays a valid OMB control number.

UTILITY PATENT APPLICATION TRANSMITTAL

(Only for new nonprovisional applications under 37 CFR 1.53(b))

UTILITY PATENT APPLICATION TRANSMITTAL		Attorney Docket No.	LEX-0092-USA
		First Inventor	C. Alexander Turner, Jr. et al.
		Title	Novel Human Secreted Proteins and Polynucleotides Encoding
		Express Mail label No.	EL672756285US

APPLICATION ELEMENTS

See MPEP chapter 600 concerning utility patent application contents.

- 1 Fee Transmittal Form (e.g., PTO/SB/17)
(Submit an original and a duplicate for fee processing)
- 2 Applicant claims small entity status.
See 37 CFR 1.27.
- 3 Specification
(preferred arrangement set forth below) 25

- Descriptive title of the Invention
- Cross Reference to Related Applications
- Statement Regarding Fed sponsored R & D
- Reference to sequence listing, a table, or a computer program listing appendix
- Background of the Invention
- Brief Summary of the Invention
- Brief Description of the drawings (if filed)
- Detailed Description
- Claim(s)
- Abstract of the disclosure

- 4 Drawing(s)(35 U.S.C.113) []
Sheets

5. Oath or Declaration-unexecuted
[Total Pages] 2

- a. Newly executed (original or copy)
- b. Copy from a prior application (37 CFR 1.63(d))
(for continuation/divisional with Box 17 completed)
- i. DELETION OF INVENTOR(S)
Signed statement attached deleting inventor(s)
named in the prior application, see 37 CFR
1.63(d)(2) and 1.33(b).

- 6 Application Data Sheet See 37 CFR 1.76

17. If a CONTINUING APPLICATION, check appropriate box, and supply the requisite information below and in a preliminary amendment, or in an Application Data Sheet under 37 CFR 1.76:

Continuation Divisional Continuation-in-part (CIP) of prior application No.: _____
Prior application information: Examiner: _____ Group/Art Unit: _____

For CONTINUATION or DIVISIONAL APPS only: The entire disclosure of the prior application, from which an oath or declaration is supplied under Box 5b, is considered a part of the disclosure of the accompanying continuation or divisional application and is hereby incorporated by reference.

18. CORRESPONDENCE ADDRESS

Customer Number or Bar Code Label [] or Correspondence address below
(Insert Customer Number or Bar Code Label here)

Name	24231		
Address	PATENT & TRADEMARK OFFICE		
City	State	Zip	
Country	Telephone	(281) 362-6554	Fax (281) 364-0155

Name (Print/Type)	<i>Lance K. Ishimoto</i>	Registration No. (Attorney/Agent)	41866
Signature	<i>[Signature]</i>	Date	November 16, 2000

Burden Hour Statement: This form is estimated to take 0.2 hours to complete. Time will vary depending upon the needs of the individual case. Any comments on the amount of time you are required to complete this form should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, Washington, DC 20231. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. SEND TO: Assistant Commissioner for Patents, Box Patent Application, Washington, DC 20231.

11/17/00 U.S. PTO
0910-0592

NOVEL HUMAN SECRETED PROTEINS AND
POLYNUCLEOTIDES ENCODING THE SAME

The present application claims the benefit of U.S.

- 5 Provisional Application Number 60/166,429 which was filed on
November 19, 1999 and is herein incorporated by reference in its
entirety.

1. INTRODUCTION

The present invention relates to the discovery,
10 identification, and characterization of novel human
polynucleotides encoding proteins that share sequence similarity
with mammalian ceruloplasmins. The invention encompasses the
described polynucleotides, host cell expression systems, the
encoded proteins, fusion proteins, polypeptides and peptides,
15 antibodies to the encoded proteins and peptides, and genetically
engineered animals that either lack or over express the disclosed
genes, antagonists and agonists of the proteins, and other
compounds that modulate the expression or activity of the proteins
encoded by the disclosed genes that can be used for diagnosis,
20 drug screening, clinical trial monitoring and the treatment of
physiological disorders.

2. BACKGROUND OF THE INVENTION

Ceruloplasmins are members of a family of metal chelating
proteins. Ceruloplasmins have been associated with development,
25 ferroxidase activity, amine oxidase activity, copper transport,
homeostasis, and superoxide dismutase activity.

3. SUMMARY OF THE INVENTION

The present invention relates to the discovery,
30 identification, and characterization of nucleotides that encode
novel human proteins, and the corresponding amino acid sequences
of these proteins. The novel human proteins (NHPs) described for

the first time herein share structural similarity with animal ceruloplasmins.

The novel human nucleic acid sequence described herein, encodes one protein/open reading frame (ORFs) 996 amino acids in 5 length (see SEQ ID NO: 2).

The invention also encompasses agonists and antagonists of the described NHP, including small molecules, large molecules, mutant NHPs, or portions thereof that compete with native NHP, peptides, and antibodies, as well as nucleotide sequences that can 10 be used to inhibit the expression of the described NHPs (e.g., antisense and ribozyme molecules, and gene or regulatory sequence replacement constructs) or to enhance the expression of the described NHP genes (e.g., expression constructs that place the described gene under the control of a strong promoter system), and 15 transgenic animals that express a NHP transgene, or "knock-outs" (which can be conditional) that do not express a functional NHP.

Further, the present invention also relates to processes for identifying compounds that modulate, i.e., act as agonists or antagonists, of NHP expression and/or NHP activity that utilize 20 purified preparations of the described NHPs and/or NHP product, or cells expressing the same. Such compounds can be used as therapeutic agents for the treatment of any of a wide variety of symptoms associated with biological disorders or imbalances.

25 4. DESCRIPTION OF THE SEQUENCE LISTING AND FIGURES

The Sequence Listing provides the sequences of the NHP ORFs encoding the described NHP amino acid sequences.

5. DETAILED DESCRIPTION OF THE INVENTION

30 The NHPs, described for the first time herein, are novel proteins that can be expressed in, *inter alia*, human testis, mammary gland and gene trapped human cell lines. The described sequences represent a synthesis of gene trapped human sequence

information and the 39 N-terminal bases of human ceruloplasmin (much of this signal sequence is cleaved from the precursor protein during secretion to produce a mature protein). The present invention encompasses the nucleotides presented in the

5 Sequence Listing, host cells expressing such nucleotides, the expression products of such nucleotides, and: (a) nucleotides that encode mammalian homologs of the described genes, including the specifically described NHPs, and the NHP products; (b) nucleotides that encode one or more portions of the NHPs that correspond to

10 functional domains, and the polypeptide products specified by such nucleotide sequences, including but not limited to the novel regions of any active domain(s); (c) isolated nucleotides that encode mutant versions, engineered or naturally occurring, of the described NHPs in which all or a part of at least one domain is

15 deleted or altered, and the polypeptide products specified by such nucleotide sequences, including but not limited to soluble proteins and peptides in which all or a portion of the signal sequence is deleted; (d) nucleotides that encode chimeric fusion proteins containing all or a portion of a coding region of an NHP, or one of its domains (e.g., a receptor or ligand binding domain, accessory protein/self-association domain, etc.) fused to another peptide or polypeptide; or (e) therapeutic or diagnostic derivatives of the described polynucleotides such as oligonucleotides, antisense polynucleotides, ribozymes, dsRNA, or

20 gene therapy constructs comprising a sequence first disclosed in the Sequence Listing. As discussed above, the present invention includes: (a) the human DNA sequences presented in the Sequence Listing (and vectors comprising the same) and additionally contemplates any nucleotide sequence encoding a contiguous NHP

25 open reading frame (ORF) that hybridizes to a complement of a DNA sequence presented in the Sequence Listing under highly stringent conditions, e.g., hybridization to filter-bound DNA in 0.5 M NaHPO₄, 7% sodium dodecyl sulfate (SDS), 1 mM EDTA at 65°C, and

washing in 0.1xSSC/0.1% SDS at 68°C (Ausubel F.M. et al., eds., 1989, Current Protocols in Molecular Biology, Vol. I, Green Publishing Associates, Inc., and John Wiley & sons, Inc., New York, at p. 2.10.3) and encodes a functionally equivalent gene product. Additionally contemplated are any nucleotide sequences that hybridize to the complement of the DNA sequence that encode and express an amino acid sequence presented in the Sequence Listing under moderately stringent conditions, e.g., washing in 0.2xSSC/0.1% SDS at 42°C (Ausubel et al., 1989, *supra*), yet still encode a functionally equivalent NHP product. Functional equivalents of a NHP include naturally occurring NHPs present in other species and mutant NHPs whether naturally occurring or engineered (by site directed mutagenesis, gene shuffling, directed evolution as described in, for example, U.S. Patent No. 5,837,458). The invention also includes degenerate nucleic acid variants of the disclosed NHP polynucleotide sequence.

Additionally contemplated are polynucleotides encoding NHP ORFs, or their functional equivalents, encoded by polynucleotide sequences that are about 99, 95, 90, or about 85 percent similar or identical to corresponding regions of the nucleotide sequences of the Sequence Listing (as measured by BLAST sequence comparison analysis using, for example, the GCG sequence analysis package using standard default settings).

The invention also includes nucleic acid molecules, preferably DNA molecules, that hybridize to, and are therefore the complements of, the described NHP gene nucleotide sequences. Such hybridization conditions may be highly stringent or less highly stringent, as described above. In instances where the nucleic acid molecules are deoxyoligonucleotides ("DNA oligos"), such molecules are generally about 16 to about 100 bases long, or about 20 to about 80, or about 34 to about 45 bases long, or any variation or combination of sizes represented therein that incorporate a contiguous region of sequence first disclosed in the

Sequence Listing. Such oligonucleotides can be used in conjunction with the polymerase chain reaction (PCR) to screen libraries, isolate clones, and prepare cloning and sequencing templates, etc.

5 Alternatively, such NHP oligonucleotides can be used as hybridization probes for screening libraries, and assessing gene expression patterns (particularly using a micro array or high-throughput "chip" format). Additionally, a series of the described NHP oligonucleotide sequences, or the complements
10 thereof, can be used to represent all or a portion of the described NHP sequences. The oligonucleotides, typically between about 16 to about 40 (or any whole number within the stated range) nucleotides in length may partially overlap each other and/or the
15 NHP sequence may be represented using oligonucleotides that do not overlap. Accordingly, the described NHP polynucleotide sequences shall typically comprise at least about two or three distinct oligonucleotide sequences of at least about 18, and preferably about 25, nucleotides in length that are each first disclosed in the described Sequence Listing. Such oligonucleotide sequences
20 may begin at any nucleotide present within a sequence in the Sequence Listing and proceed in either a sense (5'-to-3') orientation vis-a-vis the described sequence or in an antisense orientation.

For oligonucleotide probes, highly stringent conditions may
25 refer, e.g., to washing in 6xSSC/0.05% sodium pyrophosphate at 37°C (for 14-base oligos), 48°C (for 17-base oligos), 55°C (for 20-base oligos), and 60°C (for 23-base oligos). These nucleic acid molecules may encode or act as NHP gene antisense molecules, useful, for example, in NHP gene regulation (for and/or as
30 antisense primers in amplification reactions of NHP gene nucleic acid sequences). With respect to NHP gene regulation, such techniques can be used to regulate biological functions. Further,

such sequences may be used as part of ribozyme and/or triple helix sequences that are also useful for NHP gene regulation.

Inhibitory antisense or double stranded oligonucleotides can additionally comprise at least one modified base moiety which is

- 5 selected from the group including but not limited to
5-fluorouracil, 5-bromouracil, 5-chlorouracil, 5-iodouracil,
hypoxanthine, xantine, 4-acetylcytosine, 5-(carboxyhydroxymethyl)
uracil, 5-carboxymethylaminomethyl-2-thiouridine,
5-carboxymethylaminomethyluracil, dihydrouracil, beta-D-
10 galactosylqueosine, inosine, N6-isopentenyladenine,
1-methylguanine, 1-methylinosine, 2,2-dimethylguanine,
2-methyladenine, 2-methylguanine, 3-methylcytosine,
5-methylcytosine, N6-adenine, 7-methylguanine,
5-methylaminomethyluracil, 5-methoxyaminomethyl-2-thiouracil,
15 beta-D-mannosylqueosine, 5'-methoxycarboxymethyluracil,
5-methoxyuracil, 2-methylthio-N6-isopentenyladenine, uracil-5-
oxyacetic acid (v), wybutoxosine, pseudouracil, queosine,
2-thiocytosine, 5-methyl-2-thiouracil, 2-thiouracil, 4-thiouracil,
20 5-methyluracil, uracil-5-oxyacetic acid methylester, uracil-
5-oxyacetic acid (v), 5-methyl-2-thiouracil, 3-(3-amino-3-N-2-
carboxypropyl) uracil, (acp3)w, and 2,6-diaminopurine.

The antisense oligonucleotide can also comprise at least one modified sugar moiety selected from the group including but not limited to arabinose, 2-fluoroarabinose, xylulose, and hexose.

- 25 In yet another embodiment, the antisense oligonucleotide will comprise at least one modified phosphate backbone selected from the group consisting of a phosphorothioate, a phosphorodithioate, a phosphoramidothioate, a phosphoramidate, a phosphordiamidate, a methylphosphonate, an alkyl phosphotriester, and a formacetal or
30 any combination or analog thereof.

In yet another embodiment, the antisense oligonucleotide is an α -anomeric oligonucleotide. An α -anomeric oligonucleotide forms specific double-stranded hybrids with complementary RNA in

which, contrary to the usual β -units, the strands run parallel to each other (Gautier et al., 1987, Nucl. Acids Res. 15:6625-6641). The oligonucleotide is a 2'-O-methylribonucleotide (Inoue et al., 1987, Nucl. Acids Res. 15:6131-6148), or a chimeric RNA-DNA

5 analogue (Inoue et al., 1987, FEBS Lett. 215:327-330).

Alternatively, double stranded RNA can be used to disrupt the expression and function of a targeted NHP.

Oligonucleotides of the invention can be synthesized by standard methods known in the art, e.g. by use of an automated DNA 10 synthesizer (such as are commercially available from Biosearch, Applied Biosystems, etc.). As examples, phosphorothioate oligonucleotides can be synthesized by the method of Stein et al. (1988, Nucl. Acids Res. 16:3209), and methylphosphonate oligonucleotides can be prepared by use of controlled pore glass 15 polymer supports (Sarin et al., 1988, Proc. Natl. Acad. Sci. U.S.A. 85:7448-7451), etc.

Low stringency conditions are well known to those of skill in the art, and will vary predictably depending on the specific organisms from which the library and the labeled sequences are 20 derived. For guidance regarding such conditions see, for example, Sambrook et al., 1989, Molecular Cloning, A Laboratory Manual (and periodic updates thereof), Cold Springs Harbor Press, N.Y.; and Ausubel et al., 1989, Current Protocols in Molecular Biology, Green Publishing Associates and Wiley Interscience, N.Y.

25 Alternatively, suitably labeled NHP nucleotide probes can be used to screen a human genomic library using appropriately stringent conditions or by PCR. The identification and characterization of human genomic clones is helpful for identifying polymorphisms (including, but not limited to, 30 nucleotide repeats, microsatellite alleles, single nucleotide polymorphisms, or coding single nucleotide polymorphisms), determining the genomic structure of a given locus/allele, and

designing diagnostic tests. For example, sequences derived from regions adjacent to the intron/exon boundaries of the human gene can be used to design primers for use in amplification assays to detect mutations within the exons, introns, splice sites (e.g., 5 splice acceptor and/or donor sites), etc., that can be used in diagnostics and pharmacogenomics.

Further, a NHP gene homolog can be isolated from nucleic acid from an organism of interest by performing PCR using two degenerate or "wobble" oligonucleotide primer pools designed on 10 the basis of amino acid sequences within the NHP products disclosed herein. The template for the reaction may be total RNA, mRNA, and/or cDNA obtained by reverse transcription of mRNA prepared from human or non-human cell lines or tissue known or suspected to express an allele of a NHP gene.

15 The PCR product can be subcloned and sequenced to ensure that the amplified sequences represent the sequence of the desired NHP gene. The PCR fragment can then be used to isolate a full length cDNA clone by a variety of methods. For example, the amplified fragment can be labeled and used to screen a cDNA library, such as 20 a bacteriophage cDNA library. Alternatively, the labeled fragment can be used to isolate genomic clones via the screening of a genomic library.

PCR technology can also be used to isolate full length cDNA sequences. For example, RNA can be isolated, following standard 25 procedures, from an appropriate cellular or tissue source (i.e., one known, or suspected, to express a NHP gene, such as, for example, testis tissue). A reverse transcription (RT) reaction can be performed on the RNA using an oligonucleotide primer specific for the most 5' end of the amplified fragment for the 30 priming of first strand synthesis. The resulting RNA/DNA hybrid may then be "tailed" using a standard terminal transferase reaction, the hybrid may be digested with RNase H, and second strand synthesis may then be primed with a complementary primer.

Thus, cDNA sequences upstream of the amplified fragment can be isolated. For a review of cloning strategies that can be used, see e.g., Sambrook et al., 1989, *supra*.

A cDNA encoding a mutant NHP gene can be isolated, for example, by using PCR. In this case, the first cDNA strand may be synthesized by hybridizing an oligo-dT oligonucleotide to mRNA isolated from tissue known or suspected to be expressed in an individual putatively carrying a mutant NHP allele, and by extending the new strand with reverse transcriptase. The second strand of the cDNA is then synthesized using an oligonucleotide that hybridizes specifically to the 5' end of the normal gene. Using these two primers, the product is then amplified via PCR, optionally cloned into a suitable vector, and subjected to DNA sequence analysis through methods well known to those of skill in the art. By comparing the DNA sequence of the mutant NHP allele to that of a corresponding normal NHP allele, the mutation(s) responsible for the loss or alteration of function of the mutant NHP gene product can be ascertained.

Alternatively, a genomic library can be constructed using DNA obtained from an individual suspected of or known to carry a mutant NHP allele (e.g., a person manifesting a NHP-associated phenotype such as, for example, obesity, high blood pressure, an inflammatory disorder, etc.), or a cDNA library can be constructed using RNA from a tissue known, or suspected, to express a mutant NHP allele. A normal NHP gene, or any suitable fragment thereof, can then be labeled and used as a probe to identify the corresponding mutant NHP allele in such libraries. Clones containing mutant NHP gene sequences can then be purified and subjected to sequence analysis according to methods well known to those skilled in the art.

Additionally, an expression library can be constructed utilizing cDNA synthesized from, for example, RNA isolated from a tissue known, or suspected, to express a mutant NHP allele in an

individual suspected of or known to carry such a mutant allele. In this manner, gene products made by the putatively mutant tissue can be expressed and screened using standard antibody screening techniques in conjunction with antibodies raised against a normal NHP product, as described below. (For screening techniques, see, for example, Harlow, E. and Lane, eds., 1988, "Antibodies: A Laboratory Manual", Cold Spring Harbor Press, Cold Spring Harbor.)

Additionally, screening can be accomplished by screening with labeled NHP fusion proteins, such as, for example, alkaline phosphatase-NHP or NHP-alkaline phosphatase fusion proteins. In cases where a NHP mutation results in an expressed gene product with altered function (e.g., as a result of a missense or a frameshift mutation), polyclonal antibodies to a NHP are likely to cross-react with a corresponding mutant NHP gene product. Library clones detected via their reaction with such labeled antibodies can be purified and subjected to sequence analysis according to methods well known in the art.

The invention also encompasses (a) DNA vectors that contain any of the foregoing NHP coding sequences and/or their complements (i.e., antisense); (b) DNA expression vectors that contain any of the foregoing NHP coding sequences operatively associated with a regulatory element that directs the expression of the coding sequences (for example, baculovirus as described in U.S. Patent No. 5,869,336 herein incorporated by reference); (c) genetically engineered host cells that contain any of the foregoing NHP coding sequences operatively associated with a regulatory element that directs the expression of the coding sequences in the host cell; and (d) genetically engineered host cells that express an endogenous NHP gene under the control of an exogenously introduced regulatory element (i.e., gene activation). As used herein, regulatory elements include, but are not limited to, inducible and non-inducible promoters, enhancers, operators and other elements known to those skilled in the art that drive and regulate

expression. Such regulatory elements include but are not limited to the human cytomegalovirus (hCMV) immediate early gene, regulatable, viral elements (particularly retroviral LTR promoters), the early or late promoters of SV40 adenovirus, the 5 lac system, the trp system, the TAC system, the TRC system, the major operator and promoter regions of phage lambda, the control regions of fd coat protein, the promoter for 3-phosphoglycerate kinase (PGK), the promoters of acid phosphatase, and the promoters of the yeast α -mating factors.

10 The present invention also encompasses antibodies and anti-idiotypic antibodies (including Fab fragments), antagonists and agonists of the NHP, as well as compounds or nucleotide constructs that inhibit expression of a NHP gene (transcription factor inhibitors, antisense and ribozyme molecules, or gene or 15 regulatory sequence replacement constructs), or promote the expression of a NHP (e.g., expression constructs in which NHP coding sequences are operatively associated with expression control elements such as promoters, promoter/enhancers, etc.).

20 The NHPs or NHP peptides, NHP fusion proteins, NHP nucleotide sequences, antibodies, antagonists and agonists can be useful for the detection of mutant NHPs or inappropriately expressed NHPs for the diagnosis of disease. The NHP proteins or peptides, NHP fusion proteins, NHP nucleotide sequences, host cell expression systems, antibodies, antagonists, agonists and genetically 25 engineered cells and animals can be used for screening for drugs (or high throughput screening of combinatorial libraries) effective in the treatment of the symptomatic or phenotypic manifestations of perturbing the normal function of NHP in the body. The use of engineered host cells and/or animals may offer 30 an advantage in that such systems allow not only for the identification of compounds that bind to the endogenous receptor for an NHP, but can also identify compounds that trigger NHP-mediated activities or pathways.

Finally, the NHP products can be used as therapeutics (i.e., for the treatment of Wilson's Disease, etc.). For example, soluble derivatives such as NHP peptides/domains corresponding the NHPs, NHP fusion protein products (especially NHP-Ig fusion 5 proteins, i.e., fusions of a NHP, or a domain of a NHP, to an IgFc), NHP antibodies and anti-idiotypic antibodies (including Fab fragments), antagonists or agonists (including compounds that modulate or act on downstream targets in a NHP-mediated pathway) can be used to directly treat diseases or disorders. For 10 instance, the administration of an effective amount of soluble NHP, or a NHP-IgFc fusion protein or an anti-idiotypic antibody (or its Fab) that mimics the NHP could activate or effectively antagonize the endogenous NHP receptor. Soluble NHPs can also be modified by proteolytic cleavage to active peptide products (e.g., 15 any novel peptide sequence initiating at any one of the amino acids presented in the Sequence Listing and ending at any downstream amino acid). Such products or peptides can be further subject to modification such as the construction of NHP fusion proteins and/or can be derivatized by being combined with 20 pharmaceutically acceptable agents such as, but not limited to, polyethylene glycol (PEG).

Nucleotide constructs encoding such NHP products can be used to genetically engineer host cells to express such products *in vivo*; these genetically engineered cells function as "bioreactors" 25 in the body delivering a continuous supply of a NHP, a NHP peptide, or a NHP fusion protein to the body. Nucleotide constructs encoding functional NHPs, mutant NHPs, as well as antisense and ribozyme molecules can also be used in "gene 30 therapy" approaches for the modulation of NHP expression. Thus, the invention also encompasses pharmaceutical formulations and methods for treating biological disorders.

Various aspects of the invention are described in greater detail in the subsections below.

5.1 THE NHP SEQUENCES

The cDNA sequences and the corresponding deduced amino acid sequences of the described NHPs are presented in the Sequence Listing. The NHP nucleotides were obtained from human gene 5 trapped sequence tags and cDNA clones from a human mammary gland cDNA library (Edge Biosystems, Gaithersburg, MD). A coding region single nucleotide polymorphism was observed during the generation of the described NHPs which consists of a G-to-A (or vice-versa) transition at base number 1,756 of, for example, SEQ ID NO:1 which 10 results in the presence of a val or a Ile at corresponding amino acid position 586 of, for example, SEQ ID NO:2.

5.2 NHPs AND NHP POLYPEPTIDES

NHPs, polypeptides, peptide fragments, mutated, truncated, or 15 deleted forms of the NHPs, and/or NHP fusion proteins can be prepared for a variety of uses. These uses include, but are not limited to, the generation of antibodies, as reagents in diagnostic assays, for the identification of other cellular gene products related to a NHP, as reagents in assays for screening for 20 compounds that can be as pharmaceutical reagents useful in the therapeutic treatment of mental, biological, or medical disorders and disease.

The Sequence Listing discloses the amino acid sequences encoded by the described NHP encoding polynucleotides. The NHPs 25 have initiator methionine present in a DNA sequence contexts consistent with a translation initiation site, and further incorporate a hydrophobic leader sequence characteristic of secreted or membrane associated proteins.

The NHP amino acid sequences of the invention include the 30 amino acid sequence presented in the Sequence Listing as well as analogues and derivatives thereof. Further, corresponding NHP homologues from other species are encompassed by the invention. In fact, any NHP protein encoded by the NHP nucleotide sequences

described above are within the scope of the invention, as are any novel polynucleotide sequences encoding all or any novel portion of an amino acid sequence presented in the Sequence Listing. The degenerate nature of the genetic code is well known, and,

5 accordingly, each amino acid presented in the Sequence Listing, is generically representative of the well known nucleic acid "triplet" codon, or in many cases codons, that can encode the amino acid. As such, as contemplated herein, the amino acid sequences presented in the Sequence Listing, when taken together

10 with the genetic code (see, for example, Table 4-1 at page 109 of "Molecular Cell Biology", 1986, J. Darnell et al. eds., Scientific American Books, New York, NY, herein incorporated by reference)

15 are generically representative of all the various permutations and combinations of nucleic acid sequences that can encode such amino acid sequences.

The invention also encompasses proteins that are functionally equivalent to the NHPs encoded by the presently described nucleotide sequences as judged by any of a number of criteria, including, but not limited to, the ability to bind and cleave a substrate of a NHP, or the ability to effect an identical or complementary downstream pathway, or a change in cellular metabolism (e.g., proteolytic activity, ion flux, tyrosine phosphorylation, etc.). Such functionally equivalent NHP proteins include, but are not limited to, additions or substitutions of

25 amino acid residues within the amino acid sequence encoded by the NHP nucleotide sequences described above, but which result in a silent change, thus producing a functionally equivalent gene product. Amino acid substitutions may be made on the basis of similarity in polarity, charge, solubility, hydrophobicity,

30 hydrophilicity, and/or the amphipathic nature of the residues involved. For example, nonpolar (hydrophobic) amino acids include alanine, leucine, isoleucine, valine, proline, phenylalanine, tryptophan, and methionine; polar neutral amino acids include

glycine, serine, threonine, cysteine, tyrosine, asparagine, and glutamine; positively charged (basic) amino acids include arginine, lysine, and histidine; and negatively charged (acidic) amino acids include aspartic acid and glutamic acid.

5 Also encompassed by the present invention are novel protein constructs engineered in such a way that they facilitate transport of the NHP to the target site, to the desired organ, across the cell membrane and/or to the nucleus where the NHP can exert its function activity. This goal may be achieved by coupling of the
10 NHP to a cytokine or other ligand that would direct the NHP to the target organ and facilitate receptor mediated transport across the membrane into the cytosol. Conjugation of NHPs to antibody molecules or their Fab fragments could be used to target cells bearing a particular epitope. Attaching the appropriate signal
15 sequence to the NHP would also transport the NHP to the desired location within the cell. Alternatively targeting of NHP or its nucleic acid sequence might be achieved using liposome or lipid complex based delivery systems. Such technologies are described in U.S. Patents Nos. 4,594,595, 5,459,127, 5,948,767 and 6,110,490
20 and their respective disclosures which are herein incorporated by reference in their entirety.

A variety of host-expression vector systems can be used to express the NHP nucleotide sequences of the invention. Where, as in the present instance, the NHP peptide or polypeptide is thought
25 to be a soluble or secreted molecule, the peptide or polypeptide can be recovered from the culture media. Such expression systems also encompass engineered host cells that express a NHP, or functional equivalent, *in situ*. Purification or enrichment of a NHP from such expression systems can be accomplished using
30 appropriate detergents and lipid micelles and methods well known to those skilled in the art. However, such engineered host cells themselves may be used in situations where it is important not only to retain the structural and functional characteristics of

the NHP, but to assess biological activity, e.g., in drug screening assays.

The expression systems that can be used for purposes of the invention include but are not limited to microorganisms such as 5 bacteria (e.g., *E. coli*, *B. subtilis*) transformed with recombinant bacteriophage DNA, plasmid DNA or cosmid DNA expression vectors containing NHP nucleotide sequences; yeast (e.g., *Saccharomyces*, *Pichia*) transformed with recombinant yeast expression vectors containing NHP nucleotide sequences; insect cell systems infected 10 with recombinant virus expression vectors (e.g., baculovirus) containing NHP sequences; plant cell systems infected with recombinant virus expression vectors (e.g., cauliflower mosaic virus, CaMV; tobacco mosaic virus, TMV) or transformed with recombinant plasmid expression vectors (e.g., Ti plasmid) 15 containing NHP nucleotide sequences; or mammalian cell systems (e.g., COS, CHO, BHK, 293, 3T3) harboring recombinant expression constructs containing promoters derived from the genome of mammalian cells (e.g., metallothionein promoter) or from mammalian viruses (e.g., the adenovirus late promoter; the vaccinia virus 20 7.5K promoter).

In bacterial systems, a number of expression vectors may be advantageously selected depending upon the use intended for the NHP product being expressed. For example, when a large quantity of such a protein is to be produced for the generation of 25 pharmaceutical compositions of or containing NHP, or for raising antibodies to a NHP, vectors that direct the expression of high levels of fusion protein products that are readily purified may be desirable. Such vectors include, but are not limited, to the *E. coli* expression vector pUR278 (Ruther et al., 1983, EMBO J. 30 2:1791), in which a NHP coding sequence may be ligated individually into the vector in frame with the *lacZ* coding region so that a fusion protein is produced; pIN vectors (Inouye &

Inouye, 1985, Nucleic Acids Res. 13:3101-3109; Van Heeke & Schuster, 1989, J. Biol. Chem. 264:5503-5509); and the like. pGEX vectors (Pharmacia or American Type Culture Collection) can also be used to express foreign polypeptides as fusion proteins with 5 glutathione S-transferase (GST). In general, such fusion proteins are soluble and can easily be purified from lysed cells by adsorption to glutathione-agarose beads followed by elution in the presence of free glutathione. The PGEX vectors are designed to include thrombin or factor Xa protease cleavage sites so that the 10 cloned target gene product can be released from the GST moiety.

In an insect system, *Autographa californica* nuclear polyhedrosis virus (AcNPV) is used as a vector to express foreign sequences. The virus grows in *Spodoptera frugiperda* cells. A NHP coding sequence may be cloned individually into non-essential 15 regions (for example the polyhedrin gene) of the virus and placed under control of an AcNPV promoter (for example the polyhedrin promoter). Successful insertion of NHP coding sequence will result in inactivation of the polyhedrin gene and production of non-occluded recombinant virus (i.e., virus lacking the 20 proteinaceous coat coded for by the polyhedrin gene). These recombinant viruses are then used to infect *Spodoptera frugiperda* cells in which the inserted sequence is expressed (e.g., see Smith et al., 1983, J. Virol. 46: 584; Smith, U.S. Patent No. 4,215,051).

25 In mammalian host cells, a number of viral-based expression systems can be utilized. In cases where an adenovirus is used as an expression vector, the NHP nucleotide sequence of interest may be ligated to an adenovirus transcription/translation control complex, e.g., the late promoter and tripartite leader sequence. 30 This chimeric sequence may then be inserted in the adenovirus genome by in vitro or in vivo recombination. Insertion in a non-essential region of the viral genome (e.g., region E1 or E3) will result in a recombinant virus that is viable and capable of

expressing a NHP product in infected hosts (e.g., See Logan & Shenk, 1984, Proc. Natl. Acad. Sci. USA 81:3655-3659). Specific initiation signals may also be required for efficient translation of inserted NHP nucleotide sequences. These signals include the 5 ATG initiation codon and adjacent sequences. In cases where an entire NHP sequence or cDNA, including its own initiation codon and adjacent sequences, is inserted into the appropriate expression vector, no additional translational control signals may be needed. However, in cases where only a portion of a NHP coding 10 sequence is inserted, exogenous translational control signals, including, perhaps, the ATG initiation codon, must be provided. Furthermore, the initiation codon must be in phase with the reading frame of the desired coding sequence to ensure translation of the entire insert. These exogenous translational control 15 signals and initiation codons can be of a variety of origins, both natural and synthetic. The efficiency of expression may be enhanced by the inclusion of appropriate transcription enhancer elements, transcription terminators, etc. (See Bittner *et al.*, 1987, Methods in Enzymol. 153:516-544).

20 In addition, a host cell strain may be chosen that modulates the expression of the inserted sequences, or modifies and processes the gene product in the specific fashion desired. Such modifications (e.g., glycosylation) and processing (e.g., cleavage) of protein products may be important for the function of 25 the protein. Different host cells have characteristic and specific mechanisms for the post-translational processing and modification of proteins and gene products. Appropriate cell lines or host systems can be chosen to ensure the correct modification and processing of the foreign protein expressed. To 30 this end, eukaryotic host cells which possess the cellular machinery for proper processing of the primary transcript, glycosylation, and phosphorylation of the gene product may be used. Such mammalian host cells include, but are not limited to,

CHO, VERO, BHK, HeLa, COS, MDCK, 293, 3T3, WI38, and in particular, human cell lines.

For long-term, high-yield production of recombinant proteins, stable expression is preferred. For example, cell lines which 5 stably express the NHP sequences described above can be engineered. Rather than using expression vectors which contain viral origins of replication, host cells can be transformed with DNA controlled by appropriate expression control elements (e.g., promoter, enhancer sequences, transcription terminators, 10 polyadenylation sites, etc.), and a selectable marker. Following the introduction of the foreign DNA, engineered cells may be allowed to grow for 1-2 days in an enriched media, and then are switched to a selective media. The selectable marker in the recombinant plasmid confers resistance to the selection and allows 15 cells to stably integrate the plasmid into their chromosomes and grow to form foci which in turn can be cloned and expanded into cell lines. This method may advantageously be used to engineer cell lines which express the NHP product. Such engineered cell lines may be particularly useful in screening and evaluation of 20 compounds that affect the endogenous activity of the NHP product.

A number of selection systems may be used, including but not limited to the herpes simplex virus thymidine kinase (Wigler, et al., 1977, Cell 11:223), hypoxanthine-guanine phosphoribosyltransferase (Szybalska & Szybalski, 1962, Proc. 25 Natl. Acad. Sci. USA 48:2026), and adenine phosphoribosyltransferase (Lowy, et al., 1980, Cell 22:817) genes can be employed in tk⁻, hgprt⁻ or aprt⁻ cells, respectively. Also, antimetabolite resistance can be used as the basis of selection for the following genes: dhfr, which confers resistance to 30 methotrexate (Wigler, et al., 1980, Natl. Acad. Sci. USA 77:3567; O'Hare, et al., 1981, Proc. Natl. Acad. Sci. USA 78:1527); gpt, which confers resistance to mycophenolic acid (Mulligan & Berg, 1981, Proc. Natl. Acad. Sci. USA 78:2072); neo, which confers

resistance to the aminoglycoside G-418 (Colberre-Garapin, et al., 1981, *J. Mol. Biol.* 150:1); and hygro, which confers resistance to hygromycin (Santerre, et al., 1984, *Gene* 30:147).

Alternatively, any fusion protein can be readily purified by utilizing an antibody specific for the fusion protein being expressed. For example, a system described by Janknecht et al. allows for the ready purification of non-denatured fusion proteins expressed in human cell lines (Janknecht, et al., 1991, *Proc. Natl. Acad. Sci. USA* 88:8972-8976). In this system, the sequence of interest is subcloned into a vaccinia recombination plasmid such that the sequence's open reading frame is translationally fused to an amino-terminal tag consisting of six histidine residues. Extracts from cells infected with recombinant vaccinia virus are loaded onto Ni^{2+} -nitriloacetic acid-agarose columns and histidine-tagged proteins are selectively eluted with imidazole-containing buffers.

5.3 ANTIBODIES TO NHP PRODUCTS

Antibodies that specifically recognize one or more epitopes of a NHP, or epitopes of conserved variants of a NHP, or peptide fragments of a NHP are also encompassed by the invention. Such antibodies include but are not limited to polyclonal antibodies, monoclonal antibodies (mAbs), humanized or chimeric antibodies, single chain antibodies, Fab fragments, $\text{F}(\text{ab}')_2$ fragments, fragments produced by a Fab expression library, anti-idiotypic (anti-Id) antibodies, and epitope-binding fragments of any of the above.

The antibodies of the invention may be used, for example, in the detection of NHP in a biological sample and may, therefore, be utilized as part of a diagnostic or prognostic technique whereby patients may be tested for abnormal amounts of NHP. Such antibodies may also be utilized in conjunction with, for example, compound screening schemes for the evaluation of the effect of

test compounds on expression and/or activity of a NHP sequence product. Additionally, such antibodies can be used in conjunction gene therapy to, for example, evaluate the normal and/or engineered NHP-expressing cells prior to their introduction into 5 the patient. Such antibodies may additionally be used as a method for the inhibition of abnormal NHP activity. Thus, such antibodies may, therefore, be utilized as part of treatment methods.

For the production of antibodies, various host animals may be 10 immunized by injection with the NHP, an NHP peptide (e.g., one corresponding the a functional domain of an NHP), truncated NHP polypeptides (NHP in which one or more domains have been deleted), functional equivalents of the NHP or mutated variant of the NHP. 15 Such host animals may include but are not limited to pigs, rabbits, mice, goats, and rats, to name but a few. Various adjuvants may be used to increase the immunological response, depending on the host species, including but not limited to Freund's adjuvant (complete and incomplete), mineral salts such as aluminum hydroxide or aluminum phosphate, surface active 20 substances such as lysolecithin, pluronics polyols, polyanions, peptides, oil emulsions, and potentially useful human adjuvants such as BCG (bacille Calmette-Guerin) and *Corynebacterium parvum*. Alternatively, the immune response could be enhanced by 25 combination and or coupling with molecules such as keyhole limpet hemocyanin, tetanus toxoid, diphtheria toxoid, ovalbumin, cholera toxin or fragments thereof. Polyclonal antibodies are heterogeneous populations of antibody molecules derived from the sera of the immunized animals.

Monoclonal antibodies, which are homogeneous populations of 30 antibodies to a particular antigen, can be obtained by any technique which provides for the production of antibody molecules by continuous cell lines in culture. These include, but are not limited to, the hybridoma technique of Kohler and Milstein, (1975,

Nature 256:495-497; and U.S. Patent No. 4,376,110), the human B-cell hybridoma technique (Kosbor et al., 1983, Immunology Today 4:72; Cole et al., 1983, Proc. Natl. Acad. Sci. USA 80:2026-2030), and the EBV-hybridoma technique (Cole et al., 1985, Monoclonal Antibodies And Cancer Therapy, Alan R. Liss, Inc., pp. 77-96). Such antibodies may be of any immunoglobulin class including IgG, IgM, IgE, IgA, IgD and any subclass thereof. The hybridoma producing the mAb of this invention may be cultivated in vitro or in vivo. Production of high titers of mAbs in vivo makes this the presently preferred method of production.

In addition, techniques developed for the production of "chimeric antibodies" (Morrison et al., 1984, Proc. Natl. Acad. Sci., 81:6851-6855; Neuberger et al., 1984, Nature, 312:604-608; Takeda et al., 1985, Nature, 314:452-454) by splicing the genes from a mouse antibody molecule of appropriate antigen specificity together with genes from a human antibody molecule of appropriate biological activity can be used. A chimeric antibody is a molecule in which different portions are derived from different animal species, such as those having a variable region derived from a murine mAb and a human immunoglobulin constant region. Such technologies are described in U.S. Patents Nos. 6,075,181 and 5,877,397 and their respective disclosures which are herein incorporated by reference in their entirety.

Alternatively, techniques described for the production of single chain antibodies (U.S. Patent 4,946,778; Bird, 1988, Science 242:423-426; Huston et al., 1988, Proc. Natl. Acad. Sci. USA 85:5879-5883; and Ward et al., 1989, Nature 334:544-546) can be adapted to produce single chain antibodies against NHP sequence products. Single chain antibodies are formed by linking the heavy and light chain fragments of the Fv region via an amino acid bridge, resulting in a single chain polypeptide.

Antibody fragments which recognize specific epitopes may be generated by known techniques. For example, such fragments

include, but are not limited to: the $F(ab')_2$ fragments which can be produced by pepsin digestion of the antibody molecule and the Fab fragments which can be generated by reducing the disulfide bridges of the $F(ab')_2$ fragments. Alternatively, Fab expression 5 libraries may be constructed (Huse et al., 1989, *Science*, 246:1275-1281) to allow rapid and easy identification of monoclonal Fab fragments with the desired specificity.

Antibodies to a NHP can, in turn, be utilized to generate anti-idiotype antibodies that "mimic" a given NHP, using 10 techniques well known to those skilled in the art. (See, e.g., Greenspan & Bona, 1993, *FASEB J* 7(5):437-444; and Nissinoff, 1991, *J. Immunol.* 147(8):2429-2438). For example antibodies which bind to a NHP domain and competitively inhibit the binding of NHP to its cognate receptor can be used to generate anti-idiotypes that 15 "mimic" the NHP and, therefore, bind and activate or neutralize a receptor. Such anti-idiotypic antibodies or Fab fragments of such anti-idiotypes can be used in therapeutic regimens involving a NHP-mediated pathway.

The present invention is not to be limited in scope by the 20 specific embodiments described herein, which are intended as single illustrations of individual aspects of the invention, and functionally equivalent methods and components are within the scope of the invention. Indeed, various modifications of the invention, in addition to those shown and described herein will 25 become apparent to those skilled in the art from the foregoing description. Such modifications are intended to fall within the scope of the appended claims. All cited publications, patents, and patent applications are herein incorporated by reference in their entirety.

WHAT IS CLAIMED IS:

1. An isolated nucleic acid molecule comprising at least 24 contiguous bases of nucleotide sequence first disclosed 5 in the NHP polynucleotide described in SEQ ID NO:1.
2. An isolated nucleic acid molecule comprising a nucleotide sequence that:
 - (a) encodes the amino acid sequence shown in SEQ ID 10 NO:2; and
 - (b) hybridizes under stringent conditions to the nucleotide sequence of SEQ ID NO: 1 or the complement thereof.
3. An isolated nucleic acid molecule comprising a nucleotide sequence that encodes the amino acid sequence shown in SEQ ID NO:2.

09214883-111600

ABSTRACT

Novel human polynucleotide and polypeptide sequences are disclosed that can be used in therapeutic, diagnostic, and pharmacogenomic applications.

09744863, 1.1.1600

PATENT APPLICATION**DECLARATION AND POWER OF ATTORNEY
FOR PATENT APPLICATION**

ATTORNEY DOCKET NO. LEX-0092-USA

As a below named inventor, I hereby declare that:

My residence/post office address and citizenship are as stated below next to my name;

I believe I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought on the invention entitled:

Novel Human Secreted Proteins and Polynucleotides Encoding the Same

the specification of which is attached hereto unless the following box is checked:

 was filed on _____ as US Application Serial No. or PCT International Application

Number _____ and was amended on _____ (if applicable).

I hereby state that I have reviewed and understood the contents of the above-identified specification, including the claims, as amended by any amendment(s) referred to above. I acknowledge the duty to disclose all information which is material to patentability as defined in 37 CFR 1.56.

Foreign Application(s) and/or Claim of Foreign Priority

I hereby claim foreign priority benefits under Title 35, United States Code Section 119 of any foreign application(s) for patent or inventor(s) certificate listed below and have also identified below any foreign application for patent or inventor(s) certificate having a filing date before that of the application on which priority is claimed:

COUNTRY	APPLICATION NUMBER	DATE FILED	PRIORITY CLAIMED UNDER 35 U.S.C. 119
			YES: <input type="checkbox"/> NO: <input type="checkbox"/>

Provisional Application

I hereby claim the benefit under Title 35, United States Code Section 119(e) of any United States provisional application(s) listed below:

APPLICATION SERIAL NUMBER	FILING DATE
60/166,429	11/19/1999

U.S. Priority Claim

I hereby claim the benefit under Title 35, United States Code, Section 120 of any United States application(s) listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in the prior United States application in the manner provided by the first paragraph of Title 35, United States Code Section 112, I acknowledge the duty to disclose material information as defined in Title 37, Code of Federal Regulations, Section 1.56(a) which occurred between the filing date of the prior application and the national or PCT international filing date of this application:

APPLICATION SERIAL NUMBER	FILING DATE	STATUS(patented/pending/abandoned)
		.
		.
		.
		.

POWER OF ATTORNEY:

As a named inventor, I hereby appoint the following attorney(s) and/or agent(s) listed below to prosecute this application and transact all business in the Patent and Trademark Office connected therewith.

Lance K. Ishimoto, Reg. No. 41866

Send Correspondence to:

Lance K. Ishimoto
 Lexicon Genetics Incorporated
 4000 Research Forest Drive
 The Woodlands, TX 77381

Direct Telephone Calls To:

Lance K. Ishimoto
 (281) 362-6554

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

**DECLARATION AND POWER OF ATTORNEY
FOR PATENT APPLICATION (continued)**

ATTORNEY DOCKET NO. LEX-0092-USA

Full Name of Inventor: C. Alexander Turner, Jr.

Citizenship: USA

Residence: 67 Winter Wheat Place, The Woodlands, TX 77381

Post Office Address: Same

Inventor's Signature

Date

Full Name of Inventor: Brian Mathur

Citizenship: USA

Residence: 12000 Sawmill Road #2014, The Woodlands, TX 77380

Post Office Address: Same

Inventor's Signature

Date

DO NOT FILE THIS DOCUMENT
WITH THE USPTO

SEQUENCE LISTING

<110> Turner, C. Alexander Jr
Mathur, Brian

<120> Novel Human Secreted Proteins and Polynucleotides Encoding the Same

<130> LEX-0092-USA

<150> US 60/166,429
<151> 1999-11-19

<160> 2

<170> FastSEQ for Windows Version 4.0

<210> 1

<211> 2991

<212> DNA

<213> *Homo sapiens*

<400> 1

60
120
180
240
300
360
420
480
540
600
660
720
780
840
900
960
1020
1080
1140
1200
1260
1320
1380
1440
1500
1560
1620
1680
1740
1800

acagactgcc	aaggctcaa	taagatgtac	tccataatagt	gatacatgt	tggaaatctg	1860
cctggattgg	acacgtctt	aggagacaa	gttttggg	acgttttt	tgtaggatca	1920
gtggaagatt	tacacggat	atattttca	gaaataact	tcactttt	aggagcaaga	1980
agggacacat	ttccatgtt	tccttatact	tctcagacg	ttttgatgac	acctgattct	2040
ataggaactt	ttgatgggt	ttgcatgaca	ataaaagaca	atcttaggagg	catgaacat	2100
aaatatcagc	tgaggcaat	tggaaagc	aaccctgatc	aaacacaata	ccaggaggag	2160
aaaataattt	ttaccattgc	agccgaggaa	atggaaatgg	attatttetc	tagtagaaag	2220
tgggagaatg	aactccacca	cttacgaga	gagaacaaa	cgagcatgt	tgtggacaga	2280
agtggAACAC	ttcttgggtc	caaatacaag	aaagtcttat	atcgtcaata	tgtgataac	2340
acgttcacaa	atcaaacaac	aaggaaatgg	ggtagaaaaac	atctcgat	actaggctca	2400
ttaatattgc	tcaaccctgg	tcaataattt	caaattatct	ttaaaaataa	agccgcaga	2460
ccgttattca	ttcatgtca	tggagtggaa	acaataattt	ccactgtgt	tccaactcag	2520
ccaggagaga	ttcaataat	tactttgcag	atactcgata	gaactgttcc	taacctcactg	2580
gacttgcatt	gcatacttgc	ttttactat	tcaactgtat	ctgtggctaa	ggaccttcc	2640
agtggactgg	tagccctct	ctctgtatgc	cgcaaaagaca	tcaacccca	catatgttca	2700
cgtgttctcc	acttcatgtat	atttgtatgg	aatgaatctt	ggtaacttgc	agacagtatc	2760
aacacatgtt	cttcacaaacc	aaacaaacgt	gaaaggaaa	atgataat	tcaactcagc	2820
aacaaatgtc	acgcaattaa	cggaaatgt	tttggaaata	accaaaggat	acattccat	2880
gttggggatg	tagtgaatttgc	gtatctgatt	ggcataggg	atgaagctga	cctgcacaca	2940
gttcaacttca	atggccatag	ctttgaatac	aagcataataat	atttgtatg	a	2991

<210> 2
<211> 996
<212> PRT
<213> Homo sapiens

<400> 2						
Met Lys Ile Leu Ile Leu Gly Ile Phe Leu Phe Leu Cys Ser Ser Pro						
1	5	10	15			
Gly Trp Ala Ile Asp Arg His Cys Tyr Ile Gly Ile Glu Glu Ser Ile						
20	25	30				
Trp Asn Tyr Ala Pro Ser Gly Lys Asn Met Leu Asn Glu Lys Pro Phe						
35	40	45				
Ser Glu Asp Leu Glu Phe Leu Gln Gly Gln Ala Arg Lys Ser Phe						
50	55	60				
Val Phe Lys Lys Ala Leu Tyr Phe Gln Tyr Thr Asp Asn Thr Phe Gln						
65	70	75	80			
Arg Ile Ile Glu Lys Pro Ser Trp Leu Gly Phe Leu Gly Pro Met Ile						
85	90	95				
Lys Ala Glu Thr Gly Asp Phe Ile Tyr Val His Val Lys Asn Asn Ala						
100	105	110				
Ser Arg Ala Tyr Ser Tyr His Pro His Gly Leu Thr Tyr Ser Lys Glu						
115	120	125				
Asn Glu Ala His Gly Ala Ile Tyr Pro Asp Asn Thr Thr Gly Leu Gln						
130	135	140				
Lys Glu Val Glu Tyr Leu Glu Pro Gly Lys Gln Tyr Thr Tyr Lys Trp						
145	150	155	160			
Tyr Val Glu Glu His Gln Gly Pro Gly Pro Asn Asp Ser Asn Cys Val						
165	170	175				
Thr Arg Ile Tyr His Ser His Ile Asp Thr Ala Arg Asp Val Ala Ser						
180	185	190				
Gly Leu Ile Gly Pro Ile Leu Thr Cys Lys Arg Gly Thr Leu Asn Gly						
195	200	205				
Asp Thr Glu Lys Asp Ile Asp Arg Ser Ser Phe Leu Met Phe Ser Thr						
210	215	220				

Thr Asp Glu Ser Arg Ser Trp Tyr Ser Asp Glu Asn Ile Arg Ala Phe
 225 230 235 240
 Thr Glu Ser Gly Lys Ile Asn Thr Ser Asp Pro Arg Phe Glu Glu Ser
 245 250 255
 Met Ser Met Gln Ser Ile Asn Gly Tyr Ile Tyr Gly Asn Leu Pro Asn
 260 265 270
 Leu Thr Met Cys Ala Glu Asp Arg Val Gln Trp Tyr Phe Val Gly Met
 275 280 285
 Gly Gly Val Ala Asp Ile His Pro Val Tyr Leu Arg Gly Gln Thr Leu
 290 295 300
 Ile Ser Arg Asn His Arg Lys Asp Thr Ile Met Leu Phe Pro Ser Ser
 305 310 315 320
 Leu Glu Asp Ala Phe Met Val Ala Lys Ala Pro Gly Val Trp Met Leu
 325 330 335
 Gly Cys Gln Ile His Glu Ser Met Gln Ala Phe Phe Lys Val Ser Asn
 340 345 350
 Cys Gln Lys Pro Ser Thr Glu Ala Phe Val Thr Gly Thr His Val Ile
 355 360 365
 His Tyr Tyr Ile Ala Ala Lys Glu Ile Leu Trp Asn Tyr Ala Pro Ser
 370 375 380
 Gly Ile Asp Phe Phe Thr Lys Lys Asn Leu Thr Ala Ala Gly Ser Lys
 385 390 395 400
 Ser Gln Leu Phe Phe Glu Arg Ser Pro Thr Arg Ile Gly Gly Thr Tyr
 405 410 415
 Lys Lys Leu Ile Tyr Arg Glu Tyr Thr Asp Ala Ser Phe Gln Thr Gln
 420 425 430
 Lys Ala Arg Glu Glu His Leu Gly Ile Leu Gly Pro Val Phe Lys Ala
 435 440 445
 Glu Val Gly Gln Thr Ile Lys Ile Thr Phe Tyr Asn Asn Ala Ser Leu
 450 455 460
 Pro Leu Ser Ile Gln Pro Pro Gly Leu His Tyr Asn Lys Ser Asn Glu
 465 470 475 480
 Gly Leu Phe Tyr Glu Thr Pro Gly Gly Ser Thr Pro Pro Pro Ser Ser
 485 490 495
 His Val Ser Pro Gly Thr Thr Phe Val Tyr Thr Trp Glu Val Pro Lys
 500 505 510
 Asp Val Gly Pro Thr Ser Thr Asp Pro Asn Cys Leu Thr Trp Phe Tyr
 515 520 525
 Tyr Ser Ser Val Asn Gly Lys Lys Asp Ile Asn Ser Gly Leu Leu Gly
 530 535 540
 Pro Leu Leu Ile Cys Arg Asn Gly Ser Leu Gly Asp Asp Gly Lys Gln
 545 550 555 560
 Lys Gly Val Asp Lys Glu Phe Tyr Leu Leu Ala Thr Ile Phe Asp Glu
 565 570 575
 Asn Glu Ser Asn Leu Leu Asp Glu Asn Ile Arg Thr Phe Ile Thr Glu
 580 585 590
 Pro Glu Asn Ile Asp Lys Glu Asp Thr Asp Cys Gln Ala Ser Asn Lys
 595 600 605
 Met Tyr Ser Ile Asn Gly Tyr Met Tyr Gly Asn Leu Pro Gly Leu Asp
 610 615 620
 Thr Cys Leu Gly Asp Asn Val Leu Trp His Val Phe Ser Val Gly Ser
 625 630 635 640
 Val Glu Asp Leu His Gly Ile Tyr Phe Ser Gly Asn Thr Phe Thr Ser
 645 650 655
 Leu Gly Ala Arg Arg Asp Thr Ile Pro Met Phe Pro Tyr Thr Ser Gln

660 665 670
 Thr Leu Leu Met Thr Pro Asp Ser Ile Gly Thr Phe Asp Leu Val Cys
 675 680 685
 Met Thr Ile Lys His Asn Leu Gly Gly Met Lys His Lys Tyr His Val
 690 695 700
 Arg Gln Cys Gly Lys Pro Asn Pro Asp Gln Thr Gln Tyr Gln Glu Glu
 705 710 715 720
 Lys Ile Ile Ile Thr Ile Ala Ala Glu Glu Met Glu Trp Asp Tyr Ser
 725 730 735
 Pro Ser Arg Lys Trp Glu Asn Glu Leu His His Leu Arg Arg Glu Asn
 740 745 750
 Gln Thr Ser Met Tyr Val Asp Arg Ser Gly Thr Leu Leu Gly Ser Lys
 755 760 765
 Tyr Lys Lys Val Leu Tyr Arg Gln Tyr Asp Asp Asn Thr Phe Thr Asn
 770 775 780
 Gln Thr Lys Arg Asn Glu Gly Glu Lys His Leu Asp Ile Leu Gly Pro
 785 790 795 800
 Leu Ile Leu Leu Asn Pro Gly Gln Ile Ile Gln Ile Ile Phe Lys Asn
 805 810 815
 Lys Ala Ala Arg Pro Tyr Ser Ile His Ala His Gly Val Lys Thr Asn
 820 825 830
 Asn Ser Thr Val Val Pro Thr Gln Pro Gly Glu Ile Gln Ile Tyr Thr
 835 840 845
 Trp Gln Ile Pro Asp Arg Thr Gly Pro Thr Ser Leu Asp Phe Glu Cys
 850 855 860
 Ile Pro Trp Phe Tyr Tyr Ser Thr Val Ser Val Ala Lys Asp Leu His
 865 870 875 880
 Ser Gly Leu Val Gly Pro Leu Ser Val Cys Arg Lys Asp Ile Asn Pro
 885 890 895
 Asn Ile Val His Arg Val Leu His Phe Met Ile Phe Asp Glu Asn Glu
 900 905 910
 Ser Trp Tyr Phe Glu Asp Ser Ile Asn Thr Tyr Ala Ser Lys Pro Asn
 915 920 925
 Lys Val Asp Lys Glu Asn Asp Asn Phe Gln Leu Ser Asn Gln Met His
 930 935 940
 Ala Ile Asn Gly Arg Leu Phe Gly Asn Asn Gln Gly Ile Thr Phe His
 945 950 955 960
 Val Gly Asp Val Val Asn Trp Tyr Leu Ile Gly Ile Gly Asn Glu Ala
 965 970 975
 Asp Leu His Thr Val His Phe His Gly His Ser Phe Glu Tyr Lys His
 980 985 990
 Lys Tyr Leu Ile
 995